

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows.

Please replace the paragraph at page 36, lines 2-17, with the following:

A double-stranded DNA consisting of a DNA single strand having the SP6 promoter sequence at the 5' end and a complementary DNA single strand, was prepared.

70 μ l of a reaction solution having the following composition was pored into a PCR tube.

10.7 mM tris-HCl buffer (pH 8.3)

53.6 mM potassium chloride

2.36 mM magnesium chloride

0.268 mM each of dATP, dGTP, dCTP and dTTP

0.257 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.257 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT' ATC A 3' (SEQ ID NO:2)

0.032 U/ μ l commercially available DNA-dependent DNA polymerase (Ampli Taq, tradename, Perkin Elmer)

Please replace the paragraph at page 37, lines 5-12, with the following:

Fig. 1 shows the results on the gel stained with ethidium bromide. Fig 1 clearly shows single bands of DNA of about 320 bp., indicating that preparation of a DNA having the following base sequence containing the SP6 promoter sequence (underlined) at the 5' end and a complementary strand by the above-mentioned procedure.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA A- (bases 11 to 300 in HCV
cDNA) 3' (nucleotides 1-25 of SEQ ID NO:6)

Please replace the paragraph at page 37, lines 14-27, with the following:

The optimum concentration of magnesium acetate for the present invention was studied. Reaction solutions having the following composition were prepared, and 14 μ l of the reaction solutions were pored into PCR tubes.

85.7 mM tris-acetate (pH 8.1)

16.1, 32.1 or 48.2 mM magnesium acetate

214.3 mM potassium acetate

21.4% DMSO

32.1% sorbitol

2.1 mM each of ATP, GTP, CTP and UTP

2.1 mM each of dATP, dGTP, dCTP and dTTP

214 μ g/ml BSA

0.12 mM second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 38, lines 1-8, with the following:

Then, 10 μ l of a standard DNA (10^4 copies, 10^5 copies, 10^6 copies and 10^7 copies/10 μ l) or 10 μ l of DNA-free TE buffer (10 mM tris-HCl (pH 8.0) containing 0.1 mM EDTA) as a negative control was added. The standard DNA was a double-stranded DNA consisting of a DNA strand having the following sequence and a complementary DNA strand.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA A- (bases 11 to 300 in HCV
cDNA) 3' (nucleotides 1-25 of SEQ ID NO:6)

Please replace the paragraph at page 38, lines 9-21, with the following:

The reaction solutions were overlaid with 100 μ l mineral oil and incubated at 45°C for 5 minutes. Subsequently, 5 μ l of a mixed solution of the following enzymes was added, and reaction was carried out at 45°C for 1 hour.

30 U/ μ l commercially available SP6 RNA polymerase (Takara Shuzo Co., Ltd.)

12 U/ μ l commercially available RNase inhibitor (Takara Shuzo Co., Ltd.)

Then, 0.6 μ l of a third single-stranded ~~oligo~~ oligo DNA (2.75 μ M) was added.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA
TCA CTC CCC TG 3' (SEQ ID NO:1)

Please replace the paragraph at page 39, lines 8-21, with the following:

EXAMPLE 3 Effects of potassium acetate

The optimum concentration of magnesium acetate for the present invention was studied. Reaction solutions having the following composition were prepared, and 14 μ l of the reaction solutions were pored into PCR tubes.

85.7 mM tris-acetate (pH 8.1)

28.9 mM magnesium acetate

214.3, 235.7, 257.1 or 278.6 mM potassium acetate

21.4% DMSO

32.1% sorbitol

2.1 mM each of ATP, GTP, CTP and UTP

2.1 mM each of dATP, dGTP, dCTP and dTTP

214 μ g/ml BSA

0.12 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 39, line 22-page 40, line 2, with the following:

Then, 10 μ l of a standard DNA (10^3 copies, 10^4 copies, 10^5 copies and 10^6 copies/10 μ l) or 10 μ l of DNA-free TE buffer (10 μ M tris-HCl (pH 8.0) containing 0.1 mM EDTA) as a negative control was added. The standard DNA was a double-stranded DNA consisting of a DNA strand having the following sequence and a complementary DNA strand.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA A- (bases 11 to 300 in HCV cDNA) 3' (nucleotides 1-25 of SEQ IN NO:6)

Please replace the paragraph at page 40, lines 3-15, with the following:

The reaction solutions were overlaid with 100 μ l mineral oil and incubated at 45°C for 5 minutes. Subsequently, 5 μ l of a mixed solution of the following enzymes was added, and reaction was carried out at 45°C for 1 hour.

30 U/ μ l commercially available SP6 RNA polymerase (Takara Shuzo Co., Ltd.)

12 U/ μ l commercially available RNase inhibitor (Takara Shuzo Co., Ltd.)

Then, 0.6 μ l of a third single-stranded ~~oligo~~ oligo DNA (2.75 μ M) was added.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA
TCA CTC CCC TG 3' (SEQ ID NO:1)

Please replace the paragraph at page 41, lines 5-22, with the following:

The optimum sorbitol concentration for the present invention was studied. 20 μ l of reaction solutions having the following composition were pored into PCR tubes.

60 mM tris-acetate (pH 8.1).

20.3 mM magnesium acetate

187.5 mM potassium acetate

15% DMSO

22.5, 16.8, 13.5 or 11.3% sorbitol

(final concentration: 15, 11.3, 9 or 7.5%)

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

Please replace the paragraph at page 41, line 23-page 42, line 1, with the following:

Then, 5 μ l of a standard RNA (10^3 copies, 10^4 copies, 10^5 copies or 10^6 copies/5 μ l) or 5 μ l of RNA-free TE buffer as a negative control was added. The standard RNA had the following sequence.

(sequence) 5' GAA UCA AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 43, lines 1-15, with the following:

The amount of the amplification product from various concentrations of a double-stranded DNA as the target nucleic acid was studied. A reaction solution having the following composition was prepared, and 19 μ l of the reaction solution was poured into PCR tubes.

63.2 mM tris-acetate (pH 8.1).

21.3 mM magnesium acetate

197.4 mM potassium acetate

22.5% DMSO

22.5% sorbitol

1.6 mM each of ATP, GTP, CTP and UTP

1.6 mM each of dATP, dGTP, dCTP and dTTP

157.9 μ g/ml BSA

0.055 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 43, lines 16-23, with the following:

Then, 5 μ l of a standard DNA (10^3 copies, 10^4 copies, 10^5 copies or 10^6 copies/5 μ l) or 5 μ l of RNA-free TE buffer as a negative control was added. The standard DNA was a double-stranded DNA consisting of a DNA strand having the following sequence and a complementary DNA strand.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA A- (bases 11 to 300 in HCV cDNA) 3' (nucleotides 1-25 of SEQ ID NO:6)

Please replace the paragraph at page 43, line 24-page 44, line 9, with the following:

The reaction solutions were overlaid with 100 μ l mineral oil and incubated at 45°C for 5 minutes. Subsequently, 5 μ l of a mixed solution of the following enzymes was added, and reaction was conducted at 45°C for 1 hour.

30 U/ μ l commercially available SP6 RNA polymerase (Takara Shuzo Co., Ltd.)

12 U/ μ l commercially available RNase inhibitor (Takara Shuzo Co., Ltd.)

Then, 2.75 μ l of a third single-stranded oligo DNA (2.75 μ M) was added.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA TCA CTC CCC TG 3' (SEQ ID NO:1)

Please replace the paragraph at page 45, lines 3-10, with the following:

A reaction solution having the following composition was prepared, and 7.2 μ l of the reaction solution was pored into PCR tubes.

40 mM tris-HCl buffer (pH 8.0)

4 mM magnesium chloride

1 mM dithiothreitol

1 μ M first single-stranded oligo DNA (11 mer)

(sequence) 5' GTC GCC CCCGGG GGG-AA 3' (SEQ ID NO:3)

Please replace the paragraph at page 45, lines 11-21, with the following:

Then, 1.8 μ l of RNA (133 mer, 5.7 μ M) was added, 10 minutes of heating at 65°C was followed by sudden cooling in ice-cold water. The 133 mer RNA had the following sequence.

(sequence) 5' (vector sequence) GGG AAA GCU UGC AUG CCU GCA GGU CGA
CUC UAG AGG AUC CCC GGG UAC CGA GCU CGA AUU CC (sequence from HCV) U
UGG GGG CGA CAC UCC ACC AUA GAU CAC UCC CCU GUG AGG AAC UAC UGU
CUU CAC GCA GAA AGC GUC UAG C 3' (SEQ ID NO:4)

(The sequence complementary to the 11 mer DNA is underlined.)

Please replace the paragraph at page 46, lines 9-19, with the following:

Separately, a reaction solution having the following composition was prepared, and 10.8 μ l of the reaction solution was pored into PCR tubes.

40 mM tris-acetate buffer (pH 8.1)

37 mM magnesium acetate

347 mM potassium acetate

22% sorbitol

1 mM dithiothreitol

0.9 μ M first single-stranded oligonucleic acid (11 mer)

(sequence) 5' GTC ~~CGG GGG~~ GCC CCC AA 3' (SEQ ID NO:3)

Please replace the paragraph at page 47, line 26-page 48, line 16, with the following:

The present invention was performed by using various concentrations of a target RNA, and the amplification products were examined. A reaction solution having the following composition was prepared, and 20 μ l of the reaction solution was pored into PCR tubes.

60 mM tris-acetate (pH 8.1)

20.3 mM magnesium acetate

187.5 mM potassium acetate

22.5% DMSO

22.5% sorbitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 48, lines 17-22, with the following:

Then, 5 μ l of a standard RNA (10^2 copies, 10^3 copies, 10^4 copies or 10^5 copies or 10^6 copies/5 μ l) or 5 μ l of RNA-free TE buffer as a negative control was added. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 49, line 23-page 50, line 3, with the following:

Amplification products was identified after treatment with DNase or RNase. The procedure in Example 7 was followed until the use of the enzymes in 5 μ l of TE buffer as a negative control and in 5 μ l of a standard RNA (10^5 copies or 10^6 copies/5 μ l). The standard RNA was as follows.

(sequence) 5' GAA UAC AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 50, lines 4-8, with the following:

As the standard DNA, a double stranded-DNA consisting of a DNA strand having the following sequence and a complementary strand was used.

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA A- (bases 11 to 300 in HCV cDNA)-3' (nucleotides 1-25 of SEQ ID NO:6)

Please replace the paragraph at page 51, line 13-page 52, line 3, with the following:

The time courses of DNA production and RNA production were followed. A reaction solution having the following composition was prepared, and 33 μ l of the reaction solution was pored into 10 PCR tubes

61 mM tris-acetate (pH 8.1)

20.5 mM magnesium acetate

189.2 mM potassium acetate

21.7% DMSO

12 % sorbitol

15 mM dithiothreitol

1.5 mM each of ATP, GTP , CTP and UTP

1.5 mM each of dATP, dGTP, dCTP ~~dCTP~~ and dTTP

151 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 52, lines 4-8, with the following:

Then, 8.3 μ l of a standard RNA (10^6 copies/5 μ l) or TE buffer as the negative control was pored into five tubes. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases from 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of
SEQ ID NO:7)

Please replace the paragraph at page 53, line 26-page 54, line 16, with the following:

An RNA product in reaction solutions was assayed by measuring the fluorescent signals by using a fourth single-stranded oligo RNA. Firstly, 20 μ l of a reaction solution having the following composition was pored into 30 PCR tubes.

60 mM tris-acetate (pH 8.1)

20.3 mM magnesium acetate

187.5 mM potassium acetate

22.5% DMSO

12% sorbitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

Please replace the paragraph at page 54, lines 17-22, with the following:

Then, 5 μ l of a standard RNA (10^6 copies/5 μ l) and TE buffer as a negative control were added to 15 tubes, respectively. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 55, line 19-page 56, line 9, with the following:

To 50 μ l of each mixture, 100 μ l of measuring buffer having the following composition was added.

40 mM tris-acetate (pH 8.1)

13 mM magnesium acetate

125 mM potassium acetate

10 mM dithiothreitol

2 U/ μ l RNase inhibitor

37.5 nM fourth single-stranded oligo DNA (hereinafter referred to as YO-271)

(sequence) 5' CTC GC*G GGG GCT G 3' (SEQ ID NO:5)

(* indicates the site labeled with the fluorescent intercalative dye, oxazole yellow. The sequence of bases 1 to 11 in the DNA moiety is complementary to the sequence of bases 223 to 233 in HCV cDNA. The fluorescent dye was linked to the DNA moiety as described in Nucleic Acids Research, 24(24), 4992-4997 (1996) for YO-YPF-271. For the structure of YO-271, Fig. 19 should be referred to).

Please replace the paragraph at page 57, line 23-page 58, line 16, with the following:

The resulting TdT-treated YO-271 and non-treated YO-271 were used for measurement of fluorescent signals in the present invention.

50 μ l of reaction solutions having the following composition (containing non-treated YO-271 or TdT-treated YO-271) were pored into 10 tubes, respectively.

69 mM tris-acetate (pH 8.1)

20.3 mM magnesium acetate

187.5 mM potassium acetate

21.5% DMSO

12% sorbitol

15 mM dithiothreitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

112.5 nM TdT-treated YO-271 or non-treated YO-271

Please replace the paragraph at page 58, lines 17-22, with the following:

Then, 12.5 μ l of a standard RNA (10^6 copies/5 μ l) or TE buffer as a negative control was added to five tubes, respectively. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 60, line 14-page 61, line 6, with the following:

The effects of the ddTTP-treated YO-271 prepared in Example 11 on RNA or DNA synthesis in the presence of a fourth single-stranded oligo DNA were examined. 50 μ l of a reaction solution having the following composition was pored into 10 tubes.

60 mM tri-acetate (pH 8.1)

2.3 mM magnesium acetate

187.5 mM potassium acetate

21.5% DMSO

12% sorbitol

15 mM dithiothreitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

112.5 nM TdT-treated YO-271

Please replace the paragraph at page 61, lines 7-12, with the following:

Then, 12.5 μ l of a standard RNA (10^6 copies/5 μ l) or TE buffer as a negative control was added to five tubes, respectively. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases from 11 to 300 in HCV RNA)3' (nucleotides 1-8 of

SEQ ID NO:7)

Please replace the paragraph at page 62, line 18-page 63, line 10, with the following:

RNA production was monitored by using the ddTTP-treated YO-271 prepared in Example 11 as the fourth single-stranded oligo DNA over a period of time. Firstly, 50 μ l of a reaction solution having the following composition was pored into 10 tubes.

60 nM tris-acetate (pH 8.1)

20.3 mM magnesium acetate

187.5 mM potassium acetate

21.5% DMSO

12% sorbitol

15 mM dithiothreitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA

TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

112.5 nM TdT-treated YO-271

Please replace the paragraph at page 63, lines 11-16, with the following:

Then, 12.5 μ l of a standard RNA (10^6 copies/5 μ l) or TE buffer as a negative control was added to five tubes, respectively. The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases II to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)

Please replace the paragraph at page 65, lines 2-21, with the following:

The fluorescence intensity of the ddTTP-treated YO-271 prepared in Example 11 in the presence of known concentrations of a target RNA was monitored. Firstly, 50 μ l of a reaction solution having the following composition were pored into 17 PCR tubes.

60 mM tris-acetate (pH 8.1)

20.3 mM magnesium acetate

187.5 mM potassium acetate

21.5% DMSO

12% sorbitol

15 mM dithiothreitol

1.5 mM each of ATP, GTP, CTP and UTP

1.5 mM each of dATP, dGTP, dCTP and dTTP

150 μ g/ml BSA

0.3 μ M third single-stranded oligo DNA

(sequence) 5' ATT TAG GTG ACA CTA TAG AAT ACA ACA CTC CAC CAT AGA
TCA CTC CCC TG 3' (SEQ ID NO:1)

0.3 μ M second single-stranded oligo DNA

(sequence) 5' ACT CGC AAG CAC CCT ATC A 3' (SEQ ID NO:2)

112.5 nM TdT-treated YO-271

Please replace the paragraph at page 65, line 22 - page 66, line 1, with the following:

Then, 12.5 μ l of a standard RNA (10^4 copies or 10^5 copies/5 μ l) and TE buffer as a negative control were pored into four tubes each. Separately, 12.5 μ l of the standard RNA (10^6 copies/5 μ l) was pored into five tubes.

The standard RNA had the following sequence.

(sequence) 5' GAA UAC AA- (bases 11 to 300 in HCV RNA) 3' (nucleotides 1-8 of SEQ ID NO:7)